

Serological Prevalence of Anaplasmosis in Buffaloes of Wasit Province, Iraq

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Abstract

Background and aims: In compared to other domestic field animals, rare studies have conducted in buffaloes to detect the prevalence rate of different diseases. Anaplasmosis, caused by *Anaplasma marginale*, is one of the most prevalent and important haemoparasitic diseases which result in severe economic losses in particular in milk and weight. Hence, aim of the current study is investigating the prevalence rate of subclinical persistent form of anaplasmosis in buffaloes of Wasit province using of serological method (ELISA) with estimating the association of positivity to some important risk factors. **Materials and methods:** Totally, 184 buffaloes of different ages and sexes were selected randomly from different regions in Wasit province (Iraq) during April to August (2021). All study animals were and subjected to sampling of venous blood that used to collect of sera. **Results:** Examination of sera by ELISA revealed that 38.59% were positive to anaplasmosis. According to severity of infection, there were 60.56%, 22.54% and 16.9% mild, moderate and severe infections, respectively. Furthermore analysis of positive results in association with risk factors revealed a significant variation in their values. Significantly, the higher prevalence rate of anaplasmosis was seen in buffaloes aged >4-8 years while the lowest in those aged <1 year when compared to other age groups. Regarding study regions, positive infections were increased significantly in Al-Kut when compared to other study regions; Al-Aziziyah, Al-Hay and Shaykh Sa'd. However, no significant differences were observed between females and males, but males appear to be at a higher risk of infection than females. **Conclusion:** This study represents the first one in Wasit province which revealed the relative high prevalence rate of anaplasmosis in buffaloes. ELISA can apply as a test of choice for detection of carrier infections. However, furthermore studies are needed to discover the distribution of infection in different provinces of Iraq. A study of vaccine preparation and vector control programs should be applied periodically to prevent moreover infections and to eliminate ticks and flies. Sanitary precaution is necessary during vaccination, docking, castration and surgical intervention to prevent the iatrogenic transmission of anaplasmosis.

Keywords: Tick-borne disease, *Anaplasma marginale*, Bovine diseases, ELISA,

Introduction

Anaplasmosis is an infectious disease of a gram-negative, obligate, intraerythrocytic rickettsial origin which infects different domestic and wild animals. In cattle and buffaloes, *Anaplasma marginale* and to less degree *A. central* are the main causative organisms for anaplasmosis that reported usually in tropical and subtropical areas of the world [1-3]. In clinically acute form of disease, there were severe anemia due to existence of intraerythrocytic parasitism and hemolysis in addition to weakness, high body temperature, dehydration, jaundice, and decreased milk production. Infected bulls displayed a loss of libido and aberrant sperm morphology, which is occasionally accompanied by transitory testicular degeneration, while pregnant cows might be

aborted as a result of fever or anemia [4, 5]. In subclinical form of disease observed in recovered livestock, animal may carry the parasite for the rest of their lives and act as a reservoir for unaffected calves [6]. However, acute anaplasmosis could result in severe economic losses by reducing of weight, decreased milk production, miscarriage, and high treatment costs; whereas, the chronically carriers exhibit no disease's clinical symptoms can be crucial for keeping *A. marginale* within a herd [1, 7, 8].

Anaplasmosis transmission occurs mainly by ticks that act as a biological amplifier for the organism that grows in tick salivary and intestinal epithelium, and this allowing to transmission a high number of organisms during blood meal [9, 10]. Since carriers act as a reservoir for *A. marginale* for a long time, they play an important role in transmission and prevalence of disease that varied largely based on geographical location and season [11-13]. Clinical symptoms and/or necropsy findings seen in infected animals might act as a preliminary evidence of bovine anaplasmosis in acutely infected animals but not in carriers [14]. Therefore, laboratory testing using of light microscopy for stained blood smears in addition to serological / molecular diagnostic techniques are necessary to confirm clinical diagnosis of different forms of infection [15, 16]. However, the only ways to recognize carrier animals is the using of advanced assays such as competitive enzyme-linked immunosorbent assay (cELISA) that commercially available to screening of bovine *A. marginale* through detection of serum antibodies with a sensitivity and specificity reach to 95.6% and 98.6% [1].

In Iraq, anaplasmosis in buffaloes was recorded microscopically by a few numbers of studies and serologically by only one that carried out in Al-Qadisiyah and Babylon provinces [17]. Hence, the aim of current study is serologically investigation the prevalence rate of anaplasmosis caused by *A. marginale* in buffaloes of Wasit province using ELISA with estimation the association of positivity to some important risk factors.

Materials and methods

Ethical approval

The current study was conducted under the license of the Scientific Committee of the College of Agriculture, University of Wasit (Wasit, Iraq).

Study animals and sampling

Totally, 184 buffaloes of different ages and sexes were selected randomly from different regions in Wasit province (Iraq) during April to August (2021). Each study animal was subjected to sampling 5 ml of venous blood using a disposable syringe into free-anticoagulant gel-glass tubes that transported to the laboratory and centrifuged at 5000 rpm for 5 minutes to obtain sera. The serum samples were kept into labeled Eppendorf tubes and kept frozen until be tested serologically by ELISA.

Serological examination

According to manufacturer instructions of SVANOVIR® *A. marginale*-Ab Kit (SVANOVA, Sweden), the components of cELISA kits were prepared and the serum samples were thawed at room temperature. After processing of serum samples, the optical density (OD) of positive and negative controls as well as sera were measured using the Automated Microplate Reader (BioTek,

USA), and the obtained results were used to calculate of percent positivity (PP) interpretation of results. Serologically, the serum samples were considered negatives at $PP < 25$ and positives at $PP \geq 25$. Based on intensity of OD values, the positive results were divided into three categories; mild, moderate and severe infections [18].

Statistical analysis

The collected data were analyzed statistically using the tests of Chi-square (χ^2) and Odds ratio in the GraphPad Prism Software (version 6.0.1) to estimate significant variation between intensity of infection and association between positivity and targeted risk factors. Values were represented either as percentage (%) or as Mean \pm Standard Error (M \pm SE), and differences between these values were considered significant at $P < 0.05$ [19].

Results

Among totally 184 serum samples tested by ELISA, 71 (38.59%) were positive for anaplasmosis (Figure 1). According to severity of infection, there were 43 (60.56%) mild, 16 (22.54%) moderate and 12 (16.9%) severe infections (Figure 2).

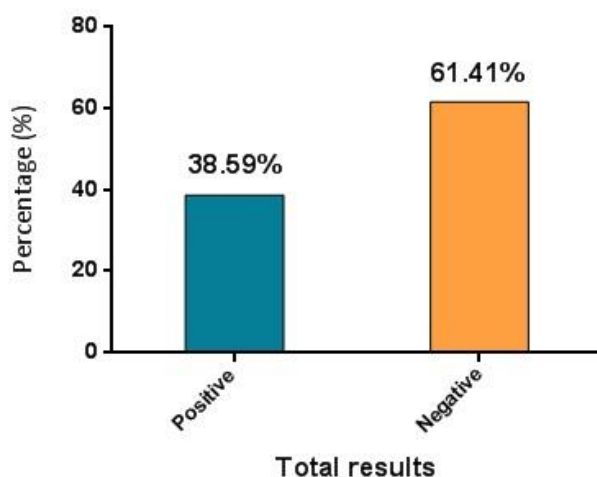


Figure (1): Total results for sera testing of 184 buffaloes by ELISA

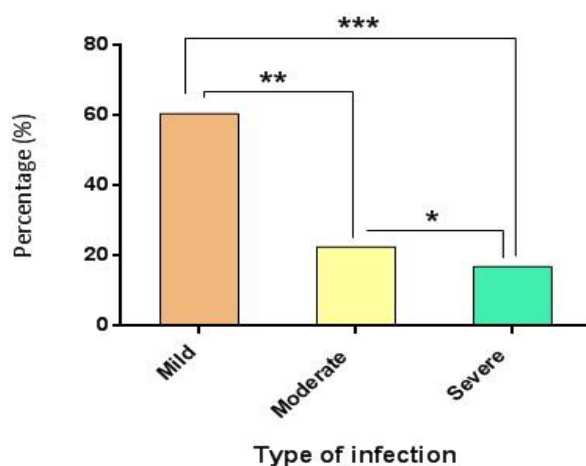


Figure (2): Severity of positive infections according to their ODs

Furthermore analysis of positive results in association with risk factors revealed a significant variation in their values (**Table 1**). Significantly, the higher prevalence rate of positive infection ($P<0.019$) was reported in buffaloes aged >4-8 years (54.43%) while the lowest one was seen in buffaloes aged <1 year (7.69%) when compared to those of 1-4 years (44.19%) and >8 years (19.44%). In addition, the findings of Odds ratio and relative risk showed that buffaloes aged >4-8 years were at a significant higher risk of infection than other age groups ($P<0.035$ and $P<0.017$, respectively). Regarding study regions, values of positive infections were increased significantly ($P<0.013$) in buffaloes of Al-Kut (58.7%) when compared to other study regions; Al-Aziziyah (30.43%), Al-Hay (41.3%) and Shaykh Sa'd (23.91%). Subsequently, values of Odds ratio and relative risk were elevated significantly ($P<0.036$ and $P<0.023$, respectively) in animals of Al-Kut rather than those of other study regions. Although, no significant differences ($P<0.064$) were observed in prevalence rate of anaplasmosis between females (37.67%) and males (42.11%), values of Odds ratio and relative risk referred that males were significantly at a higher risk of infection than females ($P<0.018$ and $P<0.015$, respectively).

Table (1): Prevalence rate of positivity among risk factors of study

Factor / Groups	Total No.	Positive No. (%)	Odds ratio	Relative risk
Age (Year)				
< 1	26	2 (7.69%)	0.107	0.176
1-4	43	19 (44.19%)	1.355	1.198
> 4-8	79	43 (54.43%) *	3.284 *	2.04 *
> 8	36	7 (19.44%)	0.294	0.449
p-value		0.019	0.035	0.017
Region				
Al-Aziziyah	46	14 (30.43%)	0.621	0.842
Al-Kut	46	27 (58.7%) *	3.036 *	1.84 *
Al-Hay	46	19 (41.3%)	1.164	1.095
Shaykh Sa'd	46	11 (23.91%)	0.408	0.549
p-value		0.013	0.036	0.023
Sex				
Female	146	55 (37.67%)	0.825	0.895
Male	38	16 (42.11%)	1.212 *	1.117 *
p-value		0.064	0.018	0.015

Significance * ($P<0.05$)

Discussion

Buffalo is one of the most important domestic animals in field which described in a number of countries as the "Black gold" [20-22]; however, few neglected studies have been conducted to detect the prevalence rate of many infectious diseases in compared to other field animals. In this study, serological investigation of anaplasmosis, known as one of the most important tick-borne pathogens worldwide, in buffaloes revealed the total infection rate was 38.59%. In comparison to

other buffalo studies in Iraq, there were 22.64% [23] and 5.71% by microscopy [24], as well as 10.33% and 36.41% by microscopy and ELISA, respectively [17]. In other countries, application of different diagnostic methods showed that the prevalence rate of anaplasmosis in buffaloes was 42.5% and 59.3% in Egypt by ELISA [25] and molecular polymerase chain reaction (PCR) assay [12], respectively; 30% in Pakistan [26]; 10.3% by PCR in Philippines [27]; 42% by reverse line blot hybridization assay in South Africa [28]; 49% and 5.4% by ELISA and PCR assay, respectively in Brazil [29]; 48.54% by ELISA and 38.72% by PCR assay in Cuba [30]; and 41% in Thailand by PCR assay [31]. This variation in prevalence rate of anaplasmosis between this study and other local and global studies may depend on differences in diagnostic methods used, environmental factors such as geographic region and type of management; animal factors such as age, sex, and breed; and number of collected samples. Therefore, these factors should carefully be evaluated when comparing the prevalence data from different studies.

In this study, high prevalence rate of mild infection in comparison with the moderate and severe infections might be explained by the usual existence of buffaloes in water might limit the direct contact with other domestic animals such as cattle as well as with the vectors. Obregón et al. (2019) supported this idea that tick-borne pathogen infection in buffaloes is influenced by local epidemiological factors including the presence of cattle in nearby regions and the common infestation by ticks [30]. The thick nature of buffalo's skin may play a role in decreasing the number of tick that come to direct contact for blood feeding. Also, to the high genetic resistance of buffaloes to different diseases was mentioned by a large number of studies [32-34]. Al-Hosary et al. (2020) mentioned that without clearing the persistent infection, buffaloes' immune system can offer protection against the high level of rickettsemia and the acute phase of the disease if the animal is challenged with the homologous strain [12].

Significant association between prevalence of anaplasmosis and age was seen in this study since higher positivity was recorded in adult young animals (> 4-8) more than other age groups; < 1, 1-4 and >8 years. These results that in agreement with many studies [17, 35, 36] might be explained by the fact that acute form of anaplasmosis occurs in younger ages of animals (1-4 years) due to frequent exposure to the sources of infection and diminishing of maternal immunity gradually between 6 months and 1 year of age. Accumulative humoral immunity in response to parasite exposure could increase the chance that adult younger animals will be found more seropositive. Older buffaloes seem to be more resistance to tick infestation due to the presence of thick skin, and then, decreasing the risk for exposure to tick-borne pathogens.

Although, all study regions are topographically identical, buffaloes of Al-Kut region were showed a higher prevalence rate of positivity in comparison with other. This could be attributed to variation in some environmental and management factors that play role in increasing of exposure to the sources of infections; infected hosts and vectors as referred by several studies [12, 18, 37, 38].

Lack of animal susceptibility to anaplasmosis according to their sex was showed in this study, and may indicate that both sexes are exposed to risk factors that increase the chance of infection. However, our results revealed that males are at higher risk of infection and this could be caused either as a result of random selection of animals since most study males were being younger in age than females or the low number of males that subjected for collection of blood and testing. However, similar findings were detected by a number of studies [12, 39, 40] but in contrast with others [26, 41, 42].

Conclusion

Relative high prevalence rate of anaplasmosis in buffaloes could refer to that the disease is extremely common in Wasit province. ELISA can apply as a test of choice for detection of carrier infections. However, furthermore studies are needed to discover the distribution of infection in different provinces of Iraq. A study of vaccine preparation and vector control programs should be applied periodically to prevent moreover infections and to eliminate ticks and flies. Sanitary precaution is necessary during vaccination, docking, castration and surgical intervention to prevent the iatrogenic transmission of anaplasmosis.

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